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Review Article

Updates of the role of oxidative stress in the pathogenesis of ovarian cancer

Ghassan M. Saed ^{a,*}, Michael P. Diamond ^b, Nicole M. Fletcher ^a^a The Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI, United States^b The Department of Obstetrics and Gynecology, Augusta University, Augusta, GA, United States

HIGHLIGHTS

- Oxidative stress plays an essential role in the pathogenesis of ovarian cancer.
- Modulating the redox balance may have therapeutic value.
- Chemoresistant ovarian cancer cells have an even further elevated oxidative stress.
- Chemotherapy-induced mutations in redox enzymes may contribute to chemoresistance.

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ABSTRACT

Clinical and epidemiological investigations have provided evidence supporting the role of reactive oxygen species (ROS) and reactive nitrogen species (RNS), collectively known as oxidative stress, in the etiology of cancer. Exogenous factors such as chronic inflammation, infection and hypoxia are major sources of cellular oxidative stress. Specifically, oxidative stress plays an important role in the pathogenesis, neoangiogenesis, and dissemination of local or distant ovarian cancer, as it is known to induce phenotypic modifications of tumor cells by cross talk between tumor cells and the surrounding stroma. Subsequently, the biological significance of the relationship between oxidative stress markers and various stages of epithelial ovarian cancer highlights potential therapeutic interventions as well as provides urgently needed early detection biomarkers. In the light of our scientific research and the most recent experimental and clinical observations, this review provides the reader with up to date most relevant findings on the role of oxidative stress in the pathogenesis of ovarian cancer and the possible therapeutic implications.

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* Corresponding author at: Wayne State University, 275 E Hancock Street, Detroit, MI 48201, United States.

E-mail address: gsaed@med.wayne.edu (G.M. Saed).

1. Ovarian cancer

Ovarian cancer is the fifth leading cause of cancer death; the leading cause of death from gynecologic malignancies, and the second most commonly diagnosed gynecologic malignancy; yet the underlying pathophysiology continues to be delineated [1]. The majority of advanced-stage tumors are of epithelial cell origin and can arise from serous, mucinous, or endometrioid cells on the surface epithelium of the ovary or the fallopian tube [1]. Surgical cytoreduction followed by platinum/taxane chemotherapy results in complete clinical response in 50–80% of patients with stage III and IV disease, but most will relapse within 18 months with chemoresistant disease [1]. Mortality rates for this type of malignancy are high because of a lack of an early-stage screening method, as well as the development of drug resistance [1].

Many cases of ovarian cancer continue to be described as de novo although several theories regarding its origination have been proposed. Some of these theories include 1) the incessant ovulation hypothesis, where ovarian surface epithelial cells are injured due to repeated ovulation leading to eventual transformation and malignancy, 2) the gonadotropin hypothesis describes overstimulation of ovarian surface epithelium through hormone receptors leading to malignant transformation, and 3) the cell of origin for most epithelial ovarian cancer is not originating in the ovary but rather coming from the fallopian tube and spreading to the ovary, and beyond [1–3]. Thus, the exact origin(s) and pathogenesis of ovarian cancer still remains under debate.

Recently, a revised model of epithelial ovarian carcinogenesis has been proposed that distinguishes more clearly between type I and type II tumors based on both molecular genetic findings and histopathologic studies [3]. Kurman and Shih describe a dualistic model of ovarian carcinogenesis where type I tumors develop from benign extraovarian precursor lesions that implant on the ovary are classified into three groups described as; endometriosis-related tumors (endometrioid, clear cell, and seromucinous), low-grade serous carcinomas, and then mucinous carcinomas and malignant Brenner tumors [3]. On the other hand, type II tumors develop from intraepithelial carcinomas in the fallopian tube, and involve both the ovary and extraovarian sites and are classified as high-grade serous carcinomas that can be further subdivided into morphologic and molecular subtypes [3].

The overwhelming majority of ovarian cancers are derived from ovarian surface epithelium. Metastasis is achieved through detachment of single cells or clusters of cells from the primary tumor followed by implantation on peritoneal mesothelial lining [4]. Unlike many other type of cancer, ovarian carcinomas rarely metastasize outside of the peritoneal cavity [5]. Additionally, the presence of spheroids in ascites is a contributing factor to not only metastasis but also to chemoresistance. Spheroid cells are also known as ovarian cancer stem cells that have numerous characteristics of cancer stem cells including self-renewal, the ability to produce differentiated progeny, increased expression of genes associated with cancer stem cells, higher invasiveness, migration potential, altered metabolism, and enhanced chemoresistance [4,6].

Ovarian cancer has also been characterized to manifest loss of function of the p53 gene due to mutations as well as other oncogenic pathways including retinoblastoma protein, the phosphatidylinositol 3 kinase (PI3K)/rat sarcoma viral oncogene pathways, and Notch signaling [1]. Moreover, ovarian cancer is associated with germline mutations in the *BRCA1* or *BRCA2* genes, affecting only 20–40% of patients, suggesting the possibility of the presence of unknown mutations in other genes [1]. Additional genetic variations, many of which have been identified in recent genome-wide association studies, have been hypothesized to act as low to moderate penetrant alleles, which contribute to ovarian cancer risk, as well as other diseases [7]. Several studies have been done to identify differentially expressed genes in ovarian carcinoma for diagnosis of early-stage ovarian cancer as well as the use of such markers as targets for improved therapy and treatment, although to date these

have not yielded reproducible prognostic indicators for identification and clinical outcomes [1,8–10].

2. Oxidative stress

The imbalance between production and elimination of free radicals and reactive metabolites leads to a state of oxidative stress and subsequent damage of important biomolecules and cells, with potential impact on the whole organism [11]. Reactive oxygen species (ROS) are oxygen-derived small molecules, including oxygen radicals, such as superoxide ($O_2^{\bullet-}$), hydroxyl (HO^{\bullet}), peroxy (RO $_2^{\bullet}$), and alkoxyl (RO $^{\bullet}$), as well as various non-radicals that can be converted to radicals or serve as oxidizing agents and include hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), ozone (O_3), and singlet oxygen (1O_2) [11,12]. Reactive nitrogen species (RNS) are nitrogen-containing oxidants and are formed from nitric oxide (NO) that is generated from the mitochondrial respiratory chain under hypoxic conditions [11]. The persistent generation of cellular ROS and RNS is a consequence of many factors including exposure to carcinogens, infection, inflammation, environmental toxicants, nutrients, and mitochondrial respiration [11–14]. Various enzyme systems produce ROS and RNS including cytochrome P450, lipoxygenase, cyclooxygenase, nicotinamide adenine dinucleotide phosphate (NAD(P)H) oxidase complex, xanthine oxidase (XO), and peroxisomes [11,13,15] (Fig. 1).

Various enzyme systems that neutralize toxic ROS and RNS are vital in maintaining the redox balance, and are summarized in Fig. 1. Superoxide dismutase (SOD) catalyzes the conversion of $O_2^{\bullet-}$ to H_2O_2 , which then can be converted to water by catalase (CAT) or glutathione peroxidase (GPX) coupled with glutathione reductase (GSR) [12] (Fig. 1). Other important scavengers include thioredoxin coupled with thioredoxin reductase, and glutaredoxin, which utilizes glutathione (GSH) as a substrate. Additionally, glutathione S-transferase (GST) is involved in detoxification of varieties of environmental carcinogens and xenobiotics by catalyzing their conjugation to GSH, and subsequent removal from the cell [12] (Fig. 1). Glutathione plays a central role in maintaining redox homeostasis, and the GSH-to-oxidized-GSH (GSH/GSSG) ratio provides an estimate of cellular redox buffering capacity [16,17]. Moreover, evidence suggests that increased oxidative stress mediated by the GSH/GSSG complex results in enhanced activity of the GS-X-MRP1 efflux pump [17]. This pump is known to decrease the intracellular effective chemotherapeutic drug concentration; therefore it is considered one of the mechanisms of multiple drug resistance [16,17].

3. Oxidative stress and cancer

Oxidative stress has been reported to affect all phases of the oncogenic process including initiation, promotion, and progression [11,12]. Oxidative stress is known to activate several transcription factors including nuclear factor (NF)- κ B, activator protein (AP)-1, p53, hypoxia inducible factor (HIF)-1 α , peroxisome proliferator-activated receptor (PPAR)- γ , β -catenin/Wnt, and Nuclear factor erythroid 2-related factor 2 (Nrf2), which modulate the expression of numerous genes involved in immune and inflammatory responses, tissue remodeling and fibrosis, carcinogenesis, and metastasis [11]. The expression of some antioxidant enzymes is known to be controlled by the master transcription factor regulator Nrf2 [11,18]. The activation of Nrf2 involves a suppressor protein known as Kelch Like ECH Associated Protein 1 (Keap1) that binds Nrf2 in the cytoplasm, preventing its translocation into the nucleus for binding specific promoters [11,18].

Reactive oxygen species are known to alter the expression of several genes through induction of genetic mutations, resulting in alteration of the balance between cell proliferation and apoptosis [1,11,19]. Damage to DNA by ROS is now accepted as a major cause of cancer, and has been demonstrated in both breast and hepatocellular carcinoma [20]. Oxidation of DNA bases, such as thymidine glycol, 5-hydroxymethyl-2'-

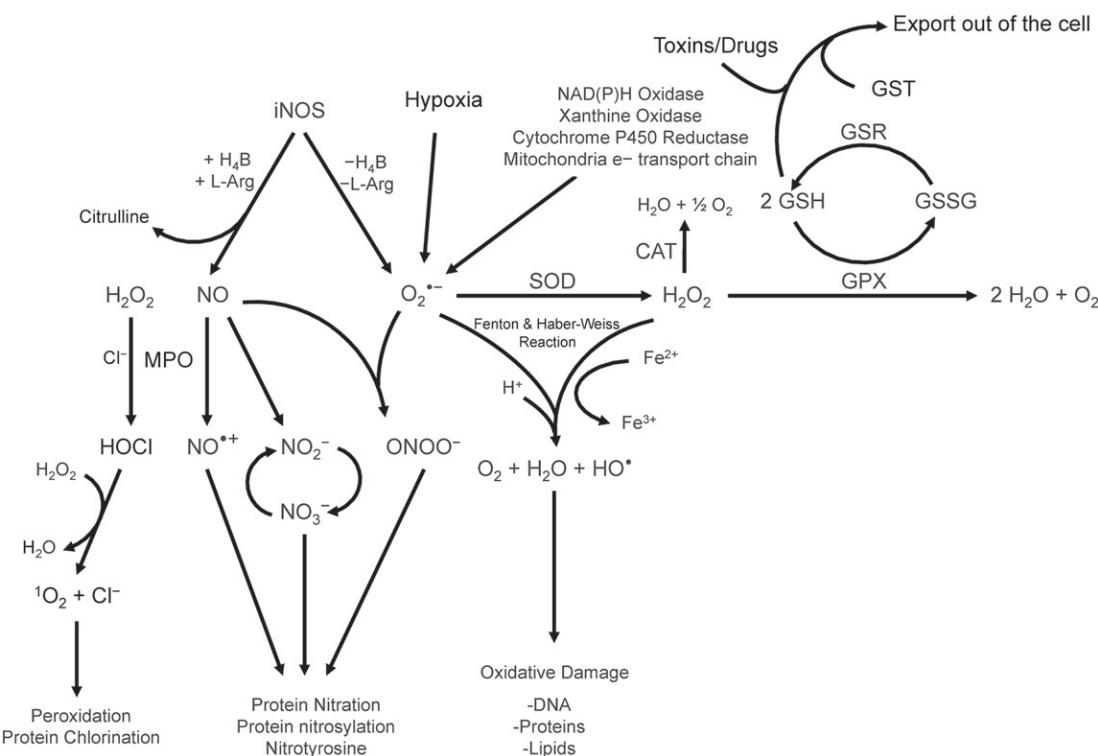


Fig. 1. Summary of key oxidant and antioxidants in cancer. Abbreviations are iNOS, inducible nitric oxide synthase; MPO, myeloperoxidase; H₂O₂, hydrogen peroxide; NO⁻, nitrite; NO₃⁻, nitrate; ONOO⁻, peroxynitrite; L-Arg, L-arginine; HO[•], hydroxyl radical; SOD, superoxide dismutase; CAT, catalase; GSH, glutathione; GPX, glutathione peroxidase; GSR, glutathione reductase; GST, glutathione S-transferase; GSSG, reduced glutathione; NAD(P)H, nicotinamide adenine dinucleotide phosphate; H₄B, tetrahydrobiopterin; Fe²⁺, Iron (II); Fe³⁺, Iron (III); O₂⁻, superoxide; NO⁺, nitrosonium cation; Cl⁻, chloride ion; HOCl, hypochlorous acid.

deoxyuridine, and 8-OHdG are now considered as markers of DNA damage by oxidative stress [19]. More importantly, ROS are considered an essential factor in the maintenance of the oncogenic phenotype by activation of certain signaling pathways, specifically, the MAPK/AP-1 and NF-κB pathways [20]. On the other hand, ROS are also required for the induction of cell death and thus can act as antitumor agents, which in this case is dependent on the concentration of ROS in the cellular environment [21].

Additionally, ROS are known to enhance tumor invasion and metastasis by increasing the rates of cell migration [1,11]. The NAD(P)H oxidase family of enzymes, a major source of cellular ROS, has been linked to the promotion of tumor cell survival and growth in pancreatic and lung cancers [1,11]. Reactive oxygen species regulate the expression of intercellular adhesion protein-1 (ICAM-1), a cell surface protein in endothelial and epithelial cells, through the activation of NF-κB. ICAM-1 and IL-8 regulate the migration of neutrophils across the endothelium, which aid in tumor metastasis [11]. Another key player in the tumor invasion process is the upregulation of specific matrix metalloproteinases (MMPs), such as MMP-2, MMP-3, MMP-9, MMP-10, and MMP-13 by H₂O₂ and NO [11]. The mechanism of MMP upregulation involves the activation of Ras, the MAPK family members ERK1/2, p38, and JNK, or the inactivation of phosphatases [11,22]. Matrix metalloproteinases are essential enzymes in the degradation of most components of the basement membrane and extracellular matrix, such as type IV collagen [11,22].

Angiogenesis is critical for the survival of solid tumors and is also regulated by ROS [11]. Angiogenesis is regulated by a number of oncogenes and tumor-suppressor genes such as Ras, c-Myc, c-Jun, mutated p53, human epidermal growth factor receptor-2, and steroid receptor coactivators through the up-regulation of VEGF or the down-regulation of thrombospondin-1 (TSP-1), an angiogenesis suppressor [11]. Reactive oxygen species stabilizes HIF-1α protein and induces the production of angiogenic factors by tumor cells.

4. Cancer cells are under intrinsic oxidative stress

Cancer cells are known to manifest increased aerobic glycolysis (Warburg effect) and high levels of intrinsic oxidative stress [23,24]. Hypoxia triggers several critical adaptations that enable cell survival: it suppresses apoptosis, alters glucose metabolism, and triggers an angiogenic phenotype [15,23]. Recent investigations suggest that O₂ depletion stimulates mitochondria to produce ROS, which subsequently activates signaling pathways, such as HIF-1α, that promote cell survival and consequently, fibrotic growth [15]. Although HIF-1α is constitutively expressed, its half-life is extremely short because it is rapidly hydroxylated by dioxygen, oxaloglutarate, and iron-dependent prolyl 4-hydroxylases (PHD 1, 2, and 3), located in the nucleus, cytoplasm, or both, respectively [24,25]. Recent studies suggest that NO and ROS, some of which may be of mitochondrial origin, can promote HIF-1α stabilization by inhibiting (prolyl hydroxylase) PHD activity [15,26]. Superoxide is converted to H₂O₂ by SOD, and the resulting H₂O₂ efflux into the cytosol inhibits PHD activity, allowing HIF-1α to accumulate, dimerize with HIF-1β, and translocate into the nucleus where it modulates the expression of genes that favor survival under hypoxic conditions [15]. Support for the role of mitochondrial ROS in HIF-1α stabilization comes from studies showing that HIF-1α stabilization can be blocked under hypoxic conditions if ROS production is abrogated in mitochondria that lack cytochrome c or that have been treated with small interfering RNA (siRNA) to knock down the Rieske protein [15,27].

Several pro-oxidant enzymes such as myeloperoxidase (MPO), inducible nitric oxide synthase (iNOS) and NAD(P)H oxidase have been found in numerous types of malignant tumors including breast, lung, prostate, bladder, colorectal and malignant melanoma, while the expression strongly depends on the histological type/grade of the tumor [9,28,29]. Similarly, antioxidants have also been associated with cancer. Both GSR and GPX expression have been reported to be differentially expressed in various types of cancer [9]. Additionally, CAT was

decreased in breast, bladder, and lung cancer while increased in brain cancer [9,28,29]. Superoxide dismutase is expressed in lung, colorectal, gastric, ovarian, and breast cancer, while decreased activity and expression have been reported in colorectal carcinomas and pancreatic cancer cells [9,28,29]. Collectively, this differential expression of oxidants and antioxidants demonstrates how the microenvironment of cancer is both unique and complex.

5. Ovarian cancer cells manifest a persistent pro-oxidant state

Oxidative stress has been implicated in the pathogenesis of several malignancies, including ovarian cancer [24,30]. Evidence suggests that ovarian cancer patients have decreased levels of circulating antioxidants and higher levels of oxidative stress [10,23,24,30–32]. In the past two decades, it has been reported that epithelial ovarian cancer (EOC) tissues and cells manifest a pro-oxidant state characterized by an increased expression of key pro-oxidant enzymes and decreased expression of antioxidant enzymes [31–33] (Table 1). Specifically, EOC cells and tissues manifested an increased expression of iNOS, MPO, NAD(P)H oxidase, as well as an increase in NO levels which correlated with expression in iNOS [31–33] (Table 1). Moreover, EOC cells manifested lower apoptosis, which was markedly induced by inhibiting iNOS with L-NAME, indicating a strong link between apoptosis and the NO/iNOS pathways in these cells [33]. More importantly, it was found that EOC cells manifested a significant increase in S-nitrosylation of caspase-3, which correlated with a significant decrease in caspase-3 activity, suggesting a potential mechanism of delayed apoptosis that was observed in these cells. Myeloperoxidase is a key oxidant enzyme that utilizes NO produced by iNOS, as a one-electron substrate generating nitrosonium cation (NO^+), a labile nitrosating species [32,34,35]. Interestingly, MPO was only recently found to be expressed by EOC cells and tissues, and has since been confirmed by other investigators [10, 32,36]. Collectively, these findings suggests that MPO is a key player in regulating apoptosis in EOC cells, but also highlights a possible cross talk between iNOS and MPO in ovarian cancer [32].

Myeloperoxidase, an abundant hemoprotein previously known to be present solely in neutrophils and monocytes, plays an essential role in immune surveillance and host defense mechanisms, and can contribute to 3-nitrotyrosine formations in vivo and directly modulates inflammatory responses via regulation of NO bioavailability during inflammation [32,37]. Silencing MPO gene expression utilizing MPO specific siRNA induced apoptosis in EOC cells through a mechanism that involved the S-nitrosylation of caspase-3 by MPO [32]. Additionally, MPO can serve as a source of free iron under oxidative stress, where both NO and O_2^- are elevated [10,32]. Iron reacts with H_2O_2 and catalyzes the generation of highly reactive hydroxyl radical (HO^\bullet), thereby increasing oxidative stress, which in turn increases free iron concentrations by the Fenton and Haber–Weiss reaction [10,32]. The potential

benefits of the combination of serum MPO and free iron as biomarkers for early detection of ovarian cancer have now been established [10]. Collectively, there is now substantial evidence demonstrating that altered oxidative stress may play a role in maintaining the oncogenic phenotype of ovarian cancer cells, and is summarized in Fig. 2.

6. Oxidative stress triggers cancer cells to favor anaerobic metabolism

Oxidative stress triggers cancer cells to favor anaerobic metabolism, despite the fact that oxygen is present [38,39]. This altered metabolism consists of an increase in glycolysis that is maintained in conditions of high oxygen tension (“aerobic glycolysis”) and gives rise to enhanced lactate production [38–40]. To compensate for the reduction in cellular ATP production, [aerobic glucose oxidation generates more ATP per glucose molecule (36 ATP) as compared to glycolysis (2 ATP)], and cancer cells upregulate glucose receptors and significantly increase glucose uptake [24,25,40]. Aerobic glycolysis, in tumor cells, results in significant lactic acidosis, which additionally induces substantial toxicity to the surrounding tissues and in cancer cells themselves. Furthermore it has been shown that lactic acidosis facilitates tumor growth, in part through breakdown of extracellular matrix, increased cell mobility/metastatic potential, and activation of angiogenesis [40]. One of the foremost nearly ubiquitous mechanisms of aerobic glycolysis resides in the activation of HIF, an oxygen-sensitive transcription factor that is activated by hypoxic stress as well as oncogenic, inflammatory, metabolic, and oxidative stress [40]. The link between oxidative stress and aerobic glycolysis is supported by the fact that HIF is activated under hypoxic conditions and is known to induce the expression of several glucose transporters as well as most of the enzymes required for glycolysis [41]. Hypoxia-inducible factor also induces the expression of pyruvate dehydrogenate kinase (PDK), an enzyme that regulates the entry of pyruvate into the mitochondria [25,40,42]. Activated PDK can inhibit pyruvate dehydrogenase (PDH), thereby limiting the entry of pyruvate into the mitochondria, where glucose oxidation can occur.

Dichloroacetate (DCA) is a metabolic modulator that has been clinically utilized in the treatment of hereditary mitochondrial diseases as well as lactic acidosis [25,43]. Dichloroacetate inhibits PDK and thus shifts glucose metabolism in cancer cells from glycolysis to glucose oxidation, reversing the unique aerobic glycolysis found in solid tumors [44]. Consistent with these findings, DCA treatment significantly decreased HIF-1 α expression [24]. Dichloroacetate has been shown to shift the oxidative balance in the intracellular redox state, leading to the activation of specific endonucleases, which induce apoptosis in EOC cells [24]. Treatment of EOC cells with DCA significantly induced apoptosis through the stimulation of caspase-3 activity in a dose-dependent manner, and was confirmed by the TUNEL assay [24]. Indeed, DCA has also been shown to induce apoptosis in cancer cells as evident by the efflux of cytochrome c and apoptosis-inducing factor from the mitochondria [45]. In support of these findings, it has been shown that aerobic glycolysis, as a result of oxidative stress, can result in resistance to apoptosis [24,46]. Several enzymes involved in glycolysis are also known to regulate apoptosis and gene transcription, suggesting that links between metabolic sensors, cell death, and gene transcription are established directly through the enzymes that control metabolism [25, 47]. Additionally, DCA induces apoptosis in glioblastoma, endometrial, prostate, and nonsmall cell lung cancers, further supporting the findings from this study, which aimed to establish a link between DCA, oxidative stress, and apoptosis in EOC cell lines, possibly through similar mechanisms [25].

Since DCA acts by activating PDH, through the inhibition of PDK, bringing pyruvate into the mitochondria and enhancing glucose oxidation, it is therefore an ideal approach to shift aerobic glycolysis to glucose oxidation coupling rather than just inhibiting aerobic glycolysis. Inhibiting aerobic glycolysis results in ATP depletion and necrosis, not apoptosis, because apoptosis is an energy-consuming process, requiring

Table 1

Summary of oxidant and antioxidant expression in sensitive and chemoresistant ovarian cancer. Abbreviations are iNOS, inducible nitric oxide synthase; MPO, myeloperoxidase; SOD, superoxide dismutase; CAT, catalase; GSH, glutathione; GPX, glutathione peroxidase; GSR, glutathione reductase; NAD(P)H, nicotinamide adenine dinucleotide phosphate.

	Ovarian cancer	Chemoresistant ovarian cancer	Reference
<i>Oxidants</i>			
MPO	↑	↑↑	[10,32,36]
iNOS	↑	↑↑	[28,32]
Nitrite/nitrate		↑↑	[9,28]
NAD(P)H oxidase	↑		[31]
<i>Antioxidants</i>			
CAT	↓	↑↑	[9,30]
GSH	↑↑	↑↑	[58]
GSR		↓↓	[9,28]
GPX		↑↑	[9]
SOD	↓	↓↓	[9,30]

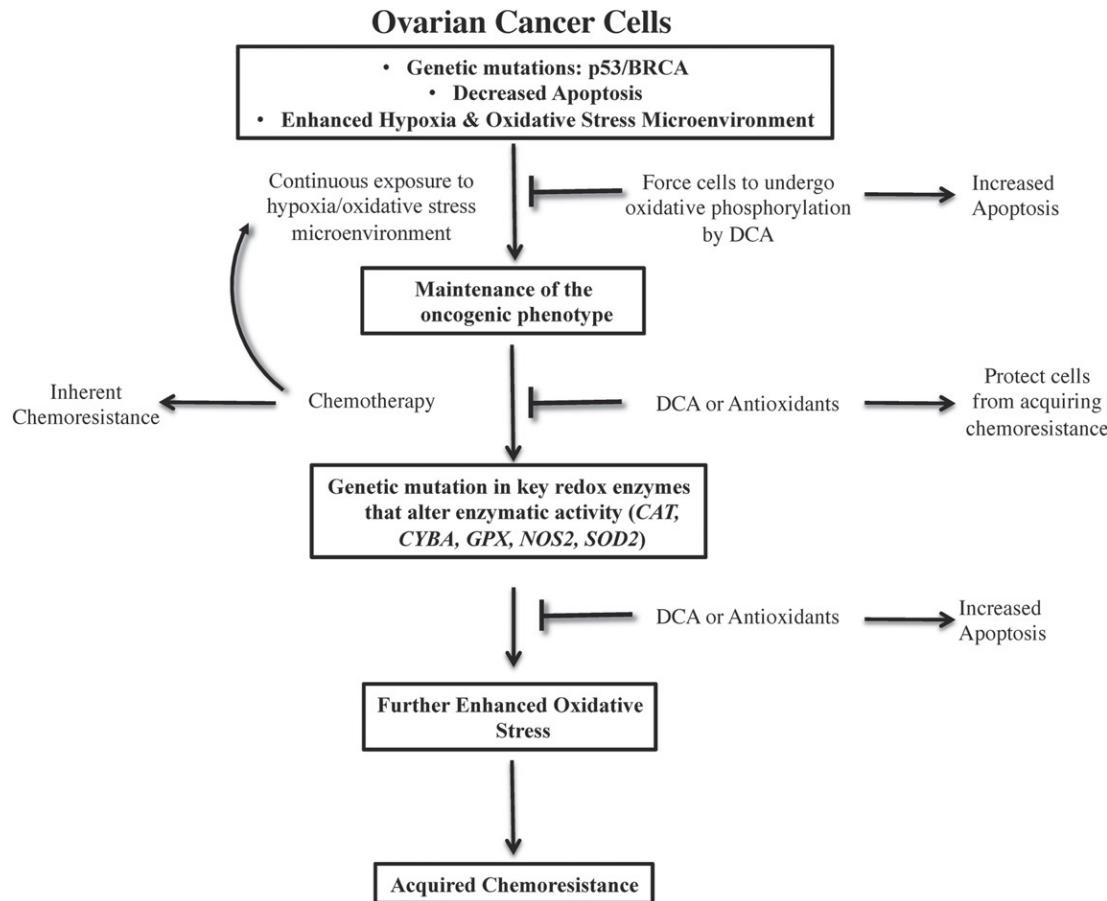


Fig. 2. Summary of the role of oxidative stress in the development of sensitive and chemoresistant ovarian cancer.

active mitochondria [25,48]. Dichloroacetate activates PDH through the inhibition of PDK at concentrations of 10 to 250 mmol/L or 0.15 to 37.5 mg/mL, in a dose-dependent fashion [25,49]. Four different isoforms of PDK have been identified that have variable expression and sensitivity to the inhibition by DCA [25,50]. Moreover, DCA administered at 35 to 50 mg/kg decreases lactate levels by more than 60% and directly activates PDH by 3- to 6-fold [25,49].

The high levels of ROS and RNS manifested by tumor cells can be countered by high levels of antioxidants, such as SOD [51]. Superoxide dismutase is considered a key antioxidant in aerobic cells and is responsible for the elimination of O_2^- by converting it to H_2O_2 . Indeed, deficiency in SOD or inhibition of the enzyme activity may cause accumulation of O_2^- in the cells, which may result in the persistence of the oncogenic phenotype [52]. Interestingly, DCA has been shown to significantly induce the expression of SOD3 in EOC cells, however, in other studies using different cancer cell lines, it was reported that decreased levels of SOD are effective in the induction of apoptosis [23,24,53]. Decreased levels of SOD may result in toxic high levels of free radicals, which ultimately could lead to necrosis. On the other hand, ROS can also induce cellular senescence and cell death and can therefore function as antitumorigenic agents [24,54]. Whether ROS promote tumor cell survival or act as antitumorigenic agents depends on the cell and tissues, the location of ROS production, and the concentration of individual ROS [11].

In summary, studies have shown that shifting anaerobic to aerobic metabolism by DCA induces apoptosis of EOC cells [24]. This effect was attributed to the modulation of key enzymes that are central to controlling the cellular redox balance. The utilization of DCA to induce apoptosis of EOC cells may provide a therapeutic option in the treatment of EOC. Explicably, the potential therapeutic value of DCA for ovarian cancer will require future analysis utilizing more cell lines, including

ovarian surface epithelial cells, fallopian tube secretory epithelial cells, as well as patients.

7. Chemotherapy and the acquisition of chemoresistance in EOC cells

Despite significant advances in surgery and anticancer treatment, chemotherapy resistance remains a major obstacle to improving a cancer patient's outcome [55]. Taxanes and platinums are the current drug therapies used for treatment of ovarian cancer. Chemoresistance greatly limits the range of possibilities for subsequent treatments, because some tumors become resistant not only to the initial drug but also to new therapeutic agents with different mechanisms of action [56]. Many chemotherapy drugs serve as a source of oxidative stress through a direct mechanism of cell death, or as an indirect effect of exposure, as observed with several chemotherapeutic agents [57]. Known factors affecting the occurrence of resistance include: altered drug influx/efflux, increased cellular GSH levels, upregulation of *Bcl-2*, decreased platinum accumulation in tumor cells, increased GSH synthesis, loss of tumor necrosis factor receptor apoptosis-inducing ligand (TRAIL)-induced apoptosis, increased DNA repair and enhanced ability to repair through up-regulation of DNA repair genes [11]. Moreover, overexpression of GST is known to reduce the reactivity of various chemotherapy drugs [58]. Additionally, loss of functional p53 augments NF- κ B activated-inflammation, thus, stabilization of wild-type p53 is critical for the prevention of EOC from progression to drug-resistance [11]. Chemoresistant EOC cells have been shown to exhibit increased expression of iNOS and nitrate/nitrite levels as well as a decrease in GSR expression, suggesting a shift towards a severe pro-oxidant state by these cells [28] (Table 1).

As mentioned earlier, EOC cells are known to manifest a pro-oxidant state characterized by increased key oxidant enzymes with concomitant decreased antioxidant enzymes [28] (Table 1). Chemotherapy resistant EOC cells are now known to also manifest an alteration in the redox balance, further advancing this pro-oxidant environment [9]. Indeed, there was a significant increase in levels of CAT, GPX, and iNOS in chemoresistant EOC cells as compared to their sensitive counterparts [9] (Table 1). In contrast, there was a decrease in levels in GSR, SOD, and the NAD(P)H oxidase subunit (p22^{phox}) in chemoresistant EOC cells [9]. This data supports an important role for an altered redox balance, not only in the maintenance of the oncogenic phenotype, but also in the development of chemoresistance (Fig. 2).

8. Polymorphisms in key oxidant and antioxidant enzymes are associated with ovarian cancer

A single nucleotide polymorphism (SNP) occurs because of point mutations that are selectively maintained in populations and are distributed throughout the human genome at an estimated overall frequency of at least one in every 1000 base pairs [59]. Recent evidence demonstrates an association between enzymatic activity altering SNPs in oxidative DNA repair genes and antioxidant genes with human cancer susceptibility [13]. Additionally, a pro-oxidant state has been implicated in the pathogenesis of several malignancies, including ovarian cancer [24,31]. This area of research is essentially reorganizing our understanding of inheritance and evolution. These modifications might explain the *in vitro* persistence of the oncogenic phenotype even after normal conditions are restored, as well as the clinical propensity for individuals to develop cancer.

This mechanism of altered enzymatic activity further explains the observation of significantly decreased apoptosis and increased survival of EOC cells [32]. Investigations into the effect of SNPs on various redox enzymes are an active area of scientific research [9,29,60,61]. The effects of genetic polymorphisms in oxidative stress-related genes on cancer susceptibility may be determined by studying functional polymorphisms in genes that control the levels of cellular ROS and oxidative damage, including SNPs for genes involved in carcinogen metabolism (detoxification and/or activation), antioxidants, and DNA repair pathways [60]. Several SNPs have been identified in key antioxidants, leading to change of function, including CAT, GPX1, GSR, and SOD2 [9, 61]. In support of this, recent studies have also associated genetic polymorphisms in genes involved in suppression of tumorigenicity as well as those involved in cell cycle with ovarian cancer [62,63]. Additional genetic variations, many of which have been identified in recent genome-wide association studies (GWAS), have been hypothesized to act as low to moderate penetrant alleles, which contribute to ovarian cancer risk, as well as other diseases [7,64].

There now is convincing evidence to suggest an association of specific SNPs in key redox enzymes with increased risk and overall survival of ovarian cancer [9,29]. Recently, a specific CAT SNP (rs1001179), that leads to reduced enzyme activity, was reported to be associated with increased risk for breast cancer and has also been described to be a significant predictor of death when present in ovarian cancer patients [9,29, 61,65]. This finding is consistent with several other studies, which linked this specific SNP with risk, response to adjuvant treatment and survival of cancer patients, including ovarian [29,66].

NAD(P)H oxidase, a key pro-oxidant enzyme, is a significant source of ROS. The membrane bound components of NAD(P)H oxidase are the catalytic subunit CYBB (gp91^{phox}) and the adjacent oxygen sensing subunit CYBA (p22^{phox}) [9,29]. Several SNPs for CYBA have been reported, some of which alter the enzyme activity. A specific SNP in CYBA (rs4673) was associated with an increased risk for ovarian cancer and other diseases where oxidative stress plays a critical role in their pathophysiology, including cardiovascular disease, asthma, and diabetic neuropathy [9,29]. The mutant genotype of the CYBA gene has been

shown to both decrease and increase activity of the protein, thereby altering the generation of O₂^{•-} [9,29].

Recent genetic studies have linked MPO to lung and ovarian cancers by demonstrating a striking correlation between the relative risk for development of the disease and the incidence of functionally distinct MPO polymorphisms [9,29]. Specifically, a SNP in *MPO* (rs2333227) was shown to be associated with increased risk for ovarian cancer [36]. Genome-wide association studies have also successfully identified and confirmed six SNPs that appear to influence the risk of EOC [9,29]. The confirmed susceptibility SNPs are rs3814113 (located at 9p22, near BNC2), rs2072590 (located at 2q31, which contains a family of HOX genes), rs2665390 (located at 3q25, intronic to TIPARP), rs10088218 (located at 8q24, 700 kb downstream of MYC), rs8170 (located at 19p13, near MERIT40), and rs9303542 (located at 17q21, intronic to SKAP1) [9,29]. Therefore, some believe that the genetic component of ovarian cancer risk may be attributed to genetic polymorphisms that confer low to moderate risk, such as SNPs that result in point mutations in the gene [67].

9. Acquisition of chemoresistance in ovarian cancer cells is associated with specific point mutations in key redox enzymes

The mechanisms underlying the acquisition of chemoresistance in ovarian cancer have yet to be fully elucidated. Evidence for an enhanced pro-oxidant state in chemoresistant EOC cells has now been described, and is thought to be a result of point mutations in key redox enzymes [9]. Specifically, a recent study observed a significant increase in levels of CAT, GPX, and iNOS while there was a significant decrease in levels of GSR, SOD, and NAD(P)H oxidase in chemoresistant EOC cells as compared to their sensitive counterparts [9]. These findings suggest a role for an altered redox balance in the development of chemoresistance in ovarian cancer. To investigate a possible mechanism of altered redox enzyme levels, the presence of several SNPs was determined in both sensitive and chemoresistant EOC cell lines. Indeed, docetaxel and/or cisplatin chemoresistant EOC cells were characterized to manifest specific point mutations, corresponding to known functional SNPs, in key redox enzymes including *SOD2* (rs4880), *NOS2* (rs2297518), and *CYBA* (rs4673) which are not present in their sensitive counterparts (Table 1). Interestingly, chemoresistant EOC cells exhibited an altered enzymatic activity for CAT and GSR while they did not exhibit the specific SNP of interest in those enzymes, which again suggests possible involvement in other functional SNPs for those enzymes (Table 1) [9]. The fact that the SNP was present in the chemoresistant EOC cells and not the sensitive cell line from which it was derived suggests that in fact, this is a point mutation rather than a SNP. To determine whether chemotherapy was capable of inducing point mutations that happen to correspond to known functional SNPs, specific point mutations in *SOD2* or *GPX1* were induced in sensitive EOC cells which led to a decrease in the sensitivity to chemotherapy, suggesting acquisition of chemoresistance [9]. Furthermore, treatment of sensitive and chemoresistant EOC cells with SOD combined with chemotherapy significantly increased the efficacy of the chemotherapy in a synergistic manner, with a more drastic effect in the chemoresistant cells [9]. This observation suggests that induction of specific point mutations in sensitive EOC cells corresponding to functional SNPs found in chemoresistant EOC cells directly reduced the sensitivity to chemotherapy (Fig. 2). These findings also support the notion that chemotherapy can induce gene point mutations that happen to correspond to SNPs in locations with functional effects, thus altering overall redox balance for survival (Fig. 2) [9].

One possible explanation for the observed nucleotide switches in response to chemotherapy is nucleotide substitution, a mechanism which includes transitions, replacement of one purine by the other or that of one pyrimidine by the other, or transversions, replacement of a purine by a pyrimidine or vice versa [9]. It has been established that hydroxyl radicals react with DNA causing the formation of a large number of

pyrimidine and purine-derived lesions [9]. The oxidative damage to 8-Oxo-2'-deoxyguanosine, an oxidized derivative of deoxyguanosine and major product of DNA oxidation, has been implicated in tumor initiation and progression through accumulation of genetic alterations of both oncogenes and tumor suppressor genes [9]. Indeed, previous findings revealed that GC → TA transversions derived from 8-hydroxy-2'-deoxyguanosine have been reported in the *ras* oncogene and the *p53* tumor suppressor gene in several cancers. It should be indicated however that GC → TA transversions are not unique to hydroxy-2'-deoxyguanosine, CC → TT substitutions have been identified as signature mutations for ROS [9].

Another explanation for the nucleotide switch is that chemoresistance resulted in an entirely different population of cells, with a new genotype. Chemotherapy eliminates the bulk of the tumor while leaving a core of cancer cells with high capacity for repair and renewal, known as cancer stem cells (CSCs) [9]. Tumors arising from CSCs usually contain a mixed population of cells due to the property of asymmetric division [9]. Cancer stem cells have been isolated from various types of cancer including leukemia, breast, brain, pancreatic, prostate, ovarian and colon [9]. Strikingly, CSCs were reported to be present in SKOV-3 EOC cells [9]. Additionally, CSCs have been shown to confer chemoresistance to cisplatin and doxorubicin in ovarian cancer cells [9].

10. Ovarian cancer immunotherapy and oxidative stress

It is well established that tumorigenic cells generate high levels of ROS to activate proximal signaling pathways that promote proliferation, survival and metabolic adaptation while also maintaining a high level of antioxidant activity to prevent buildup of ROS to levels that could induce cell death [68]. Moreover, there is evidence that ROS can act as second messengers in immune cells, which can lead to hyperactivation of inflammatory responses resulting in tissue damage and pathology [68]. Ovarian cancer is considered an ideal tumorigenic cancer because ovarian cancer cells have no negative impact on immune cells [69].

Effective immunotherapy for ovarian cancer is currently the focus of several investigations and clinical trials. Current immunotherapies for cancer treatment include therapeutic vaccines, cytokines, immune modulators, immune checkpoint inhibitors, and adoptive T cell transfer [70]. The discovery of a monoclonal antibody (bevacizumab) directed against vascular endothelial growth factor (VEGF) which has been shown to improve progression free survival compared to cytotoxic chemotherapy alone was a major outcome of clinical trials [71]. Other monoclonal antibodies currently approved for other cancers such as trastuzumab for breast cancer or cetuximab for colon cancer exhibited limited activity in ovarian cancer [71]. Several clinical trials are ongoing for the utilization of immune checkpoint blockade in ovarian cancer immune therapy [72]. Most recently tested were the programmed death (PD)-1 inhibitors, pembrolizumab and nivolumab, which showed a consistent response rate of 10–20% in phase 2 studies and then failed to improve outcomes in confirmatory trials [72]. Ultimately, larger phase 3 studies are needed to validate these findings for checkpoint inhibitors, particularly with regard to the duration of response seen with these agents. Additionally, the direct intraperitoneal delivery of interleukin (IL)-12, a potent immunostimulatory agent, exhibited some potential therapeutic efficacy in ovarian cancer [73]. Recently, targeting folate receptor alpha, which is found to be expressed in ovarian cancer, has shown promising therapeutic value. The targeting of the folate receptor was achieved by either a blocking monoclonal antibody (farletuzumab) or antibody conjugates of folate analogs, such as vintafolide [74].

11. Summary and conclusion

Oxidative stress has been implicated in the pathogenesis of several malignancies including ovarian cancer. Epithelial ovarian cancer is characterized to manifest a persistent pro-oxidant state through alteration

of the redox balance, which is further enhanced in their chemoresistant counterparts, as summarized in Table 1 and Fig. 2. Forcing ovarian cancer cells to undergo oxidative phosphorylation rather than glycolysis has been shown to be beneficial for eliminating cells via apoptosis (Fig. 2). Collectively, there is convincing evidence that indicated a causal relationship between the acquisition of chemoresistance and chemotherapy-induced genetic mutations in key redox enzymes, leading to a further enhanced oxidative stress in chemoresistant EOC cells. This concept was further confirmed by the observation that induction of point mutations in sensitive EOC cells increased their resistance to chemotherapy. Also, a combination of antioxidants with chemotherapy significantly sensitized cells to chemotherapy. Identification of targets for chemoresistance with either biomarker and/or screening potential will have a significant impact for the treatment of this disease.

Conflicts of interest

GMS and NMK disclose no potential conflicts of interest. MPD receives grant and contract support from the NIH/NICHD, Abbvie, Bayer, and PCORI/AHRQ. MPD is also a stockholder and on the Board of Directors for Advanced Reproductive Care, LLC.

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